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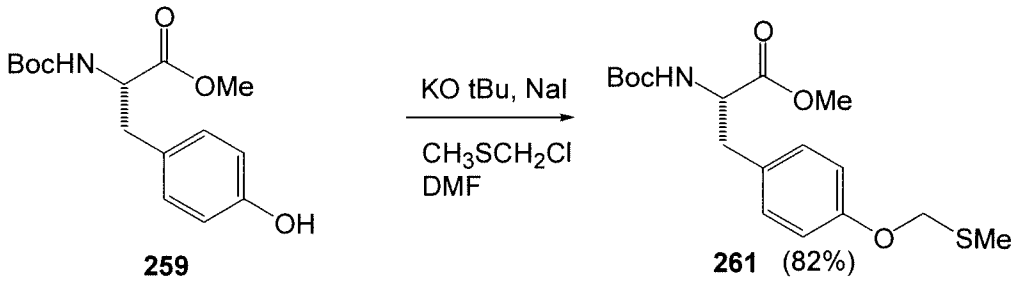
APPENDIX 1

anisaldehyde stain (18 mL p-anisaldehyde, 7.5 mL glacial acetic acid, 25 mL conc. $HySO_4$, 675 mL absolute EtOH), ninhydrin solution (200 mg ninhydrin, 95 mL n-butanol, 5 mL 10% AcOH), potassium permanganate solution (3g $KMnO_4$, 20g K_2CO_3, 5 mL 5% aqueous NaOH, 300 mL H_2O). Flash chromatography was performed on Scientific Adsorbents Incorporated silica gel (32-63 μM, 60 Å pore size) using distilled reagent grade hexanes and ACS grade ethyl acetate, methanol and chloroform. The term, "concentrated in vacuo" refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (< 20 torr), followed by residual solvent removal at high vacuum (< 0.1 torr). The term, "high vacuum," refers to vacuum achieved by a mechanical belt-drive oil pump.

All yields are reported as the average of three independent trials with the exception of the transformation of compound 271 to 270 (two trials) and peptide syntheses (one trial each). This averaging accounts for the discrepancy in yields reported in the below, representative procedures and in the text.

B. Detailed Synthetic Procedures

BocTyr(MTM)OMe:

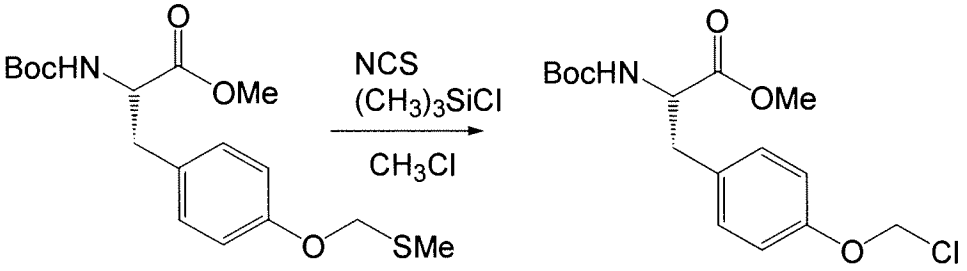


259 **261** (82%)

To a solution of BocTyrOMe (4.05 g, 13.7 mmol) and NaI (206 mg, 1.37 mmol) in DMF (30 mL) chilled via an external ice bath was added a THF (15 mL) solution of potassium *t*-butoxide (1.73 g, 15.1 mmol). To the resultant phenoxide (clear green solution) chloromethyl methyl sulfide (1.33 mL, 15.1 mmol) was added slowly. The reaction was allowed to warm gradually to room temperature. After 4.5 h, the reaction was cloudy and TLC analysis (4:1 hexanes/EtOAc) indicated complete consumption of starting material. The reaction mixture was diluted with EtOAc (60 mL) and washed with H_2O (1 x 45 mL), aqueous citric acid solution (5%, 1 x 45 mL) and brine (1 x 45 mL). The aqueous washing were pooled and washed with EtOAc (2 x 60 mL). The combined

organic extracts were pooled and dried over $MgSO_4$. The $MgSO_4$ was removed by filtration and volatiles removed *in vacuo*. The residue was purified by flash column chromatography (silica, gradient elution 4:1 hexanes/EtOAc to 2:1 hexanes/ EtOAc) to afford a clear syrup on concentration **261**(3.99 g, 81 %). R_f = .44 (4:1, hexanes/EtOAc); **IR** (Neat): 3368, 1744, 1714, 1510 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.02-6.81(AA'BB', J = 9.5, 4H), 5.06 (s, 2H), 4.99 (d, J = 5.4, 1H), 4.49 (q, J = 5.8, 1H), 3.66 (s, 3H), 2.18 (s, 3H), 1.36 (s, 9H); **^{13}C NMR** (75 MHz, CDC1y): δ 172.2, 156.0, 130.3, 129.1, 116.0, 72.3, 54.4, 52.2, 37.5, 28.3, 14.4; **LRMS** (ESI): *m/z* 378 [M+Na^+ calc'd for $C_{17}H_{25}NO_5S$ 378.1]

BocTyr(CH_2)Cl:



261 **262**

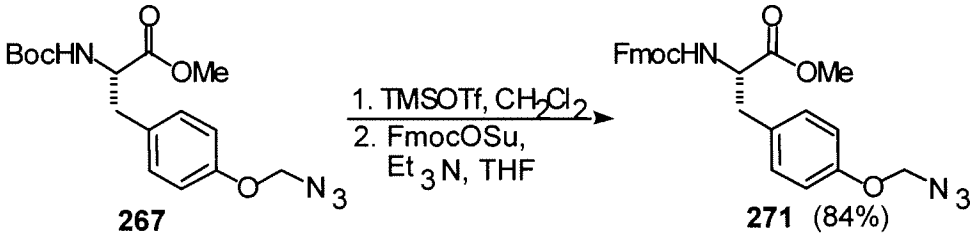
The *O,S*-acetal **261** (287 mg, 0.81 mmol) was dissolved in dichloromethane (3.0 mL), and solid NCS (119 mg, 0.89 mmol) was added. The reaction was allowed to stir for 2.5 h, then trimethylsilyl chloride (.11 mL, 0.89 mmol) was added. After an additional 2 hours, the crude reaction mixture was loaded directly on to a flash silica gel column. Elution with 5:1 hexanes/EtOAc and drying *in vacuo* provided the compound as a clear oil in pure form for characterization. The compound **262** was crystallized to yield white plates under high vacuum for 10 h (202 mg, 73%). The mass balance was recovered as BocTyrOMe after elution with EtOAc. R_f = .40 (4:1, hexanes/EtOAc); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.10-6.98 (AA'BB', J = 8.8 Hz, 4 H), 5.84 (s, 2H), 4.98 (d, J = 7.7 Hz, 1H), 4.53 (q, J = 7.8 Hz, 1H), 3.68 (s, 3H), 3.05- 2.95 (m, 2H), 1.38 (s, 9H);**^{13}C NMR** (75 MHz, $CDCl_3$): δ 172.1, 154.6, 130.4, 116.1, 54.3, 52.1, 37.4, 28.1; **LRMS** (MALDI, α-cyano-4-hydroxycinnamic acid matrix, positive ion mode): *m/z* 368.2, 366.1, 318.2 [calc'd M+Na^+ for $C_{16}H_{22}ClNO_5$ 366.11; M+Na^+ for $C_{15}H_{21}NO_5$ 318.13].

BocTyr(Azm)OMe:

261 **267** (87%)

The *O,S*-acetal **261** (4.28 g) was dissolved in CH_2Cl_2 (35 mL) and solid N-chlorosuccinimide (1.76 g) was added. The reaction was allowed to stir at room temperature for 4 h. Trimethylsilyl chloride (1.68 mL) was then added slowly. After an additional 6 h, the reaction was diluted with $CHCl_3$ (30 mL) and saturated $NaHCO_3$ solution (60 mL) was added. The organic layer was separated and the aqueous fraction was extracted with $CHCl_3$ (2 x 60 mL). The combined organic extracts were concentrated via rotary evaporation and the residue dissolved in DMF(15 mL). Sodium azide (1.2 g, 18.5 mmol) was dissolved in H_2O (15 mL) and added to the solution of crude tyrosyl chloride. This reaction was allowed to stir for 5 h at room temperature. The reaction was then diluted with saturated $NaHCO_3$ solution (15 mL) and washed with EtOAc (3 x 30 mL). The combined organic extracts were dried ($MgSO_4$) concentrated *in vacuo*, and the residue subjected to flash column chromatography (silica, gradient elution 4:1 hexanes/EtOAc to 2:1 hexanes/EtOAc). After removal of volatiles, azidomethylene **267** was isolated as a clear oil (3.64 g, 87%). $\mathbf{R_f}$ = .41 (4:1 hexanes/EtOAc); **IR** (Neat): 2132, 2110 cm '; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.08-6.90 (AA'BB', J = 8.5 Hz, 4 H), 5.13 (s, 2H), 4.95 (d, J = 6.7 Hz, 1H), 4.55 (d, J =6.7 Hz 1H), 3.71 (s, 3H), 3.0 (m, 2H), 1.41 (s, 9H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 172.1, 155.4, 130.3, 115.8, 79.6, 54.3, 51.9, 37.2, 28.0; **LRMS** (FAB): *m/z* 373.1 [M+Na^+ calc'd for $C_{16}H_{22}N_4O_5$ 373.2]

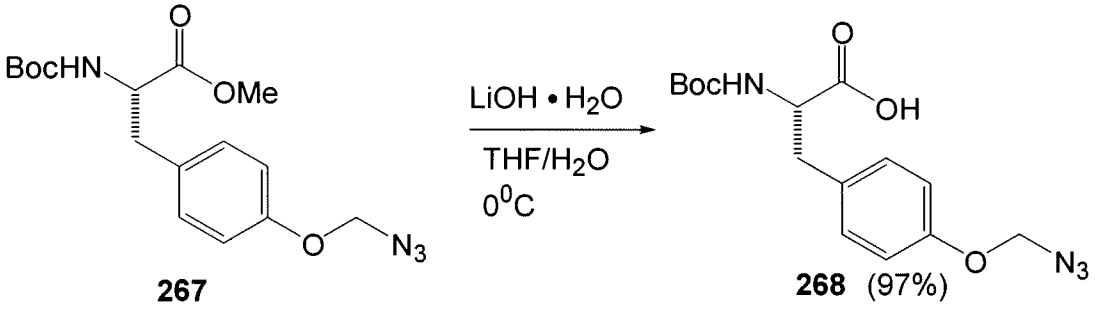
FmocTyr(Azm)OMe:



267 **271** (84%)

Boc protected compound **267** (769 mg, 2.19 mmol) was dissolved in CH_2Cl_2 and cooled with an external ice bath. TMSOTf (0.79 mL, 4.4 mmol) was added dropwise. TLC analysis (4:1 Hexanes/EtOAc) indicated complete consumption of starting material after less than 10 min. 5% aqueous Na_2CO_3 was added (15 mL), followed by EtOAc (15 mL). The organic layer was separated and the aqueous phase extracted with EtOAc (3 x 15 mL). Volatiles were removed *in vacuo* and the residue was taken up in THF (7 mL). Triethylamine (0.91 mL, 6.6 mmol) was added, followed by solid FmocOSu (814 mg, 2.4 mmol). The reaction was left to stir for 3.5 h during which time a white precipitate formed. The reaction was diluted with $CHCl_3$ (15 mL) and washed with H_2O, 5% citric acid solution, and brine (15 mL each). The combined aqueous washings were extracted with $CHCCl_3$ (4 x 15 mL). The pooled organic extracts were dried over $MgSO_4$ and filtered. The solvent was reduced to ca. 5 mL via rotary evaporation and loaded directly on a silica gel column. Flash chromatography (2:1 hexanes/EtOAc) yielded the compound **271** as a crystalline white solid (870 mg, 84%). Ry= .36 (2:1 hexanes/EtOAc); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 7.76-7.74 (d, J = 7.7 Hz, 2H, 7.57-7.53 (m, 2H), 7.41-7.36 (t, J = 7.1 Hz, 2H), 7.36-7.23 (t, J = 7.3 Hz, 2H), 7.02-6.88 (AA'BB', J = 7.4 Hz, 4H), 5.28 (d, J = 8.1 Hz, 1H), 5.09 (s, 2H), 4.63 (dd, J = 7.0, J = 10.6, 1H), 4.44 (dd, J = 7.0, J = 10.6, 1H), 4.16 (t, J = 7.0, 1H), 3.71 (s, 3H), 3.13-2.99 (m, 2H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 171.7, 155.6, 143.7, 141.2, 130.4, 129.8, 127.6, 126.9, 126.8, 124.9, 119.9, 115.9, 79.6, 66.7, 54.7, 52.2, 47.0, 37.7; **LRMS** (ESI): m/z 495.1 [M+Na^+ calc'd for $C_{26}H_{24}N_4O_5$ 495.16]

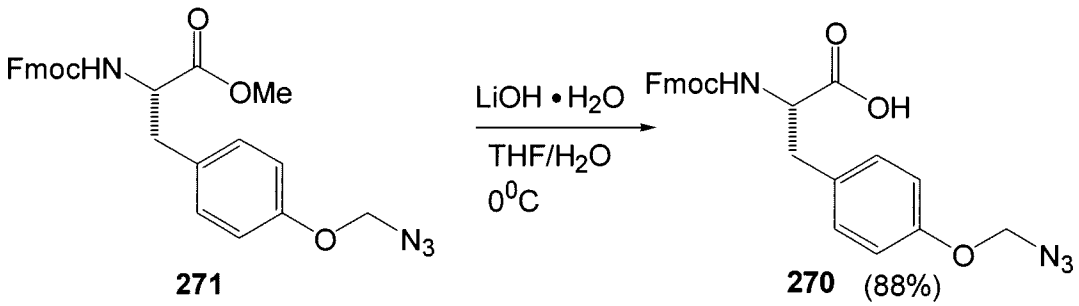
BocTyr(Azm)OH:



267 **268** (97%)

Methyl ester **267** (622 mg, 1.78 mmol) was dissolved in THF (5 mL) and cooled with an external ice bath. $LiOH{\cdot}H_2O$ (224 mg, 5.34 mmol) was dissolved in H_2O (5 mL) and chilled in the ice bath. The LiOH solution was then added to the methyl ester in one aliquot. After stirring at 0 °C for 2h TLC analysis (4:1, hexanes/EtOAc) indicated complete consumption of starting material. The reaction was diluted with 5% aqueous citric acid solution (20 mL), yielding an apparent pH of 3 (pH paper). The aqueous solution was extracted with EtOAc (4 x 20 mL). The combined organic extracts were pooled and dried over $MgSO_4$. Filtration and removal of volatiles *in vacuo* provided **268** as a white foam suitably pure (according to TLC, ^{1}H NMR) for analytical characterization (584 mg, 97%). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.75 (br s, 1H), 7.12-6.90 (AA'BB', J = 6.9 Hz, 4H), 6.45 (m, minor rotamer NH) 4.96 (d, 7.7 Hz, major rotamer NH) 4.55 (m, major rotamer αH, .61/1H), 4.33 (m, minor rotamer αH, .38/1H), 2.95-2.80 (m, major rotamer, β2H), 1.40 (s, major rotamer, *t*-Bu), 1.29 (s, minor rotamer, *t*-Bu).

FmocTyr(Azm)OH:

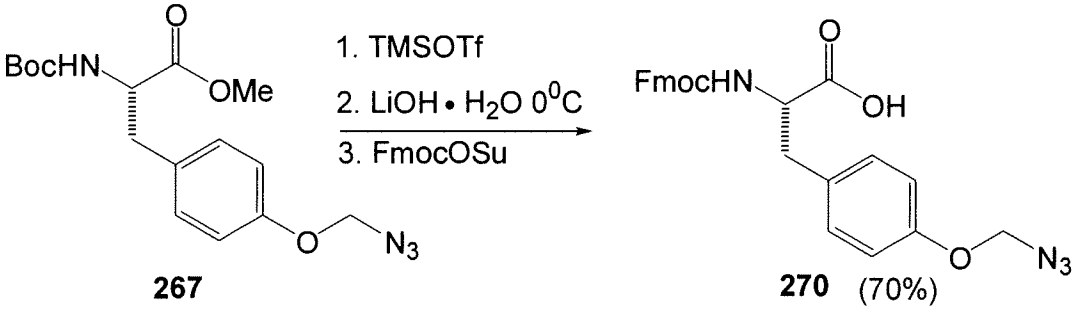


271 **270** (88%)

Methyl ester **271** (339 mg, 0.72 mmol) was dissolved in THF (7 mL) and cooled to 0 °C with an external ice bath. $LiOH{\cdot}H_2O$ (60 mg, 1.4 mmol) was dissolved in H_2O (7 mL) and added dropwise over 10 minutes to the chilled solution of methyl ester. After an

additional 25 min the starting material was completely consumed as judged by analytical TLC (2:1 hexanes/EtOAc). The pH was then adjusted to ca. 3 by adding 0.3 M aqueous HCl. The cloudy aqueous solution was extracted with EtOAc (4 x 15 mL). The organic extracts were pooled, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash silica gel chromatography (10 % MeOH/$CHCl_3$) provides **270** as a white solid after drying *in vacuo* (292 mg, 89%). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 10.05 (br s, 1H), 7.78 (d, J = 7.7 Hz, 2H), 7.59-7.52 (m, 2H), 7.41 (t, J =7.4 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 7.09-6.90 (AA'BB', J = 6.9 Hz, 4H), 6.20 (m, minor rotamer NH), 5.43 (d, J = 8.1 Hz, major rotamer NH), 5.08 (s, 2H), 4.67 (q, J = 6.6, 1H), 4.52-4.46 (m, 1H), 4.40-4.34 (m, 1H), 4.23-4.17 (m, 1H), 3.23-3.05 (m, major rotamer βH), 2.98-2.80 (m, minor rotamer βH); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 155.4, 147.8, 143.3, 140.9, 130.2, 127.4, 126.7, 126, 124.6, 79.3, 66.7, 46.7, 36.5; **LRMS** (ESI): m/z 457.1, 235.1 [MH calc'd for $C_{25}H_{22}O_5N_4$ 457.15; MH calc'd for $C_{25}H_{22}O_5N_4$ – $C_{15}H_{11}O_2$ 235.1].

FmocTyr(Azm)OH, Method B:



Boc Tyr(Azm)OMe **267** (766 mg, 2.19 mmol) was dissolved in CH_2Cl_2 (10 mL and cooled with an external ice bath. Trimethylsilyl triflate (0.79 mL, 4.4 mmol) was added dropwise over 3 minutes. After an additional 15 minutes, TLC analysis (5:1, hexanes/EtOAc) indicated complete consumption of starting material. Aqueous Na_2CO_3 solution (5%, 25 mL) was added, followed by 10 mL $CHCl_3$. The two phases were separated and the aqueous phase washed with EtOAc (4 x 25 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to give 2.1 g of an oil. This crude material was dissolved in THF (10 mL) and chilled via an external ice bath. A chilled solution of $LiOH{\cdot}H_2O$ (276 mg, 6.57 mmol) in H_2O (10 mL) was added. After 2 hours acetic acid (2.5 eq) was added and the ice bath was removed. Solid FmocOSu (740 mg,

2.19 mmol) was added, and this mixture was allowed to stir for 2 hours. The bulk of the THF was then removed via rotary evaporation and triethylamine (1 mL) was added. The crude aqueous mixture was filtered through a medium sintered glass frit and the solid washed with H_2O (3 x 15 mL). The combined aqueous mixture was acidified to an apparent pH of 2 (pH paper) with 1 N HCl and washed with EtOAc (4 x 60 mL). The combined organic extracts were concentrated and the residue purified by flash column chromatography (silica, gradient elution 95:5 $CHCl_3$/MeOH to 80:20 $CHCl_3$/MeOH). Drying *in vacuo* yields **270** as an off-white, chalky solid (678 mg, 1.53 mmol, 68%). Analytical data (TLC, ^{1}HNMR, ^{13}CNMR, LRMS) same as above.

Attachment of the C-terminal amino acid to 2-chlorotrityl chloride resin:

Amino acid is suspended in dichloromethane (DCM, 10 mL per gram of resin) and dimethylformamide (DMF) is added dropwise until the amino acid dissolves. 1.1 equivalents of diisopropylethylamine should be used relative to the total mmoles amino acid plus mmoles chloride. The amino acid is added to the resin along with 1/3 the total amount of diisopropylethylamine. After stirring with a small stir bar for five minutes the rest of the base is added, and the mixture is stirred for 1 hour. After 1 hour, 1 mL of methanol is added to cap the resin. The resin slurry is then transferred to a fritted funnel and rinsed with DCM (3X), DMF (2X), iPrOH (2X), DMF (2X), iPrOH (2X), MeOH (2X), and Et_2O (2X). Solvent volume for all washes is 8 mL per gram of resin.

The stability of the carboxylate – chlorotrityl bond is enhanced by deblocking of the α-amino group. Thus, the Fmoc group is cleaved by rinsing of the resin with 10% piperidine/CH_2Cl_2 (2x), followed by 20% piperidine/DMF for 20 minutes. The resin is agitated via sparging with nitrogen gas during this reaction. At the conclusion of the Fmoc cleavage the resin is rinsed, DCM (3X), DMF (2X), iPrOH (2X), DMF (2X), iPrOH (2X), MeOH (2X), and Et_2O (2X). Solvent volume for all washes is 8 mL per gram of resin. The resin is then dried under high vacuum and stored at sub-zero temperatures. In general, superior loadings are achieved using this protocol relative to commercially available, pre-loaded resins.

Peptide Synthesis:

Synthesis using FmocTyr(Azm)OH for the introduction of sulfotyrosine residues was carried out on 25 μM scale using an automated synthesizer (Applied Biosystems Model 432A "Synergy"). Standard techniques were used (Fields, G. B., and Noble, R. L. (1990) Solid-Phase Peptide-Synthesis Utilizing 9-Fluorenylmethoxycarbonyl Amino-Acids, International Journal of Peptide and Protein Research 35, 161-214; Merrifield, R. B. (1963)Solid Phase peptide Synthesis I, The synthesis of a Tetrapeptide, Journal of the American Chemical Society 85, 7129-7133.) The first amino acid was attached as above or the pre-loaded resins were purchased from Advanced Chemtech (Louisville, Kentucky). Carboxylic and alcoholic side chains were protected with benzyl groups. Each synthesis cycle is initiated with the cleavage of the Fmoc group from the α-amino group, using 20% piperidine in DMF. Three equivalents (75 μmol) of the amino acid to be coupled is dissolved in DMF and added to the resin cartridge with HBTU (2-(1H-benzotriazol-l-yl)-1,1,3,3 tetramethyluronium hexafluorophosphate) and HOBt (N-hydroxybenzotriazole). The reaction cartridge is subjected to continuous flow conditions during each reaction. Following the coupling of the final amino acid, the peptide – resin cartridge is removed from the synthesizer. All subsequent manipulations of the peptide – resin are performed manually. Subsequent reactions are agitated by the "double syringe" method. Briefly, a luer lock syringe is attached to each end of the peptide synthesis cartridge and the syringes are moved reciprocally and in tandem to agitate the reaction.

Fmoc-cleavage:

The resin-bound peptide is flushed with 5% piperidine/CH_2Cl_2 followed by treatment with 20% piperidine/DMF for 20 min. The piperidine solution is removed, and the resin rinsed [DCM (3X), DMF (2X), iPrOH (2X), DMF (2X)].

Acetylation:

The terminal amino group is acetylated prior to further synthetic manipulations. The peptide synthesis cartridge (PSC) is flushed with inert gas and rinsed with DMF (1mL), 2:1 pyridine/AcyO (1 mL) is then added and the acetylation reaction agitated via

the double syringe method. After three hours the resin is rinsed with 2:1 pyridine/Ac_2O (1 mL), and alternately with DMF, MeOH, CH_2Cl_2, Et_2O (3 x 1 mL each). Amine capping is confirmed by the Kaiser test (Kaiser, E. (1970). Analytical Biochemistry 34, 595).

General protocol for azidomethylene cleavage of resin-bound peptides:

To a conical flask charged with anhydrous SnC1y (57 mg 0.3 mmol) is added THF (2 mL), PhSH (104pL, 1.2 mmol) and Et_2N (170pL, ca.1.2 mmol). This mixture is stirred briefly under Ar. The dry resin (.025 mmol peptide) is flushed with THF (3 x 0.5 mL) and 1 mL of the reducing cocktail is added to the PSC. The reaction is agitated via the double syringe method. After ca. 5 minutes the reducing mixture is removed from the PSC and the resin rinsed with THF. The remainder of the reducing cocktail is added and the resin is agitated for ca. 5 minutes. The reducing cocktail is then removed and the resin washed alternately with moist THF:Et_2N (9:1, 4 x 1 mL), CH_2Cl_2 (4 x 1 mL), MeOH (4 x 1 mL) and DMF (4 x 1 mL).

Sulfation: The PSC is flushed with 4:1 DMF/pyridine (1 mL). DMF·SO_2 (115 mg, 0.75 mmol) is dissolved in 4:1 DMF/pyridine and the resultant solution added to the PSC. The sulfation reaction is agitated by the double syringe method. After eight hours the resin is rinsed with 4:1 DMF/ pyridine (1 mL) and the sulfation repeated with fresh DMF SOy for an additional eight hours. The resin is then washed alternately with 4:1 DMF/pyridine and methanol (3 x 1 mL), then DMF, CH_2Cl_2, Et_2O (3 x 1mL each).

Cleavage of sulfotyrosine peptides from 2-chlorotrityl resin:

The resin is dried under vacuum for at least 2 h prior to attempting the cleavage reaction. The dried resin is transferred to a round bottom flask and placed in an ice bath. A solution of 7:2:1 CH_2Cl_2,/TFE/AcOH is cooled in an ice bath. Cleavage solution (10 mL per gram of resin) is added and the slurry stirred with a magnetic stir bar for 1.5h. The cleavage mixture is then filtered through a sintered glass frit and the resin rinsed with three additional volumes of cleavage solution. The filtrate is then concentrated via rotary evaporation at ca. 10°C to ca. 1/3 of the original volume. 100

mM ammonium acetate buffer is added and the mixture lyophilized to dryness. The crude material is then subjected to HPLC purification.

Ac YA: This dipeptide was synthesized manually using the double syringe method. Fmoc-L-Ala was attached to 2-Clt resin according to the standard procedure. Fmoc cleavage was followed by coupling of FmocTyr(Azm) (PyBop, DIPEA) according to the method of Castro et al. Fmoc cleavage and acetylation was followed by cleavage from the support. HPLC purification [Vydac C18, gradient elution: A = 0.1%TFA/H_2O, B: CH_3CN/0.1%TFA 0 – 20% B/30min t_2 = 26.76. Excision of the peaks and weighing on an analytical balance revealed relative peak size of 92.3:7.7 (84.6% ee). The synthesis of this dipeptide was repeated using Carpino's amide bond forming conditions (HATU, HOAt, collidine). All other steps were performed in the same way. This synthesis yielded material with a relative peak size of at least 95:5 (>90% ee).

^{1}H NMR (300 MHz, CD_3OD): δ 8.35 (d, J = 6.5 Hz, amide NH), 7.10-6.69 (AB q, J = 6.4 Hz, 4H), 4.58 (dd, J = 4.3, J = 5.0, 1H), 4.43-4.36 (m, 1H), 3.11-2.73 (m, 2H), 1.90 (s, 3H), 1.41 (d, J = 7.5, 3H).

AcY-(D)-A: HPLC purification [Vydac C18, gradient elution: A = 0.1%TFA/H_2O, B: CH_3CN/ 0.1% TFA 0 – 20% B/30min t_2 = 21.4 min. Excision and weighing of the peaks gave a ratio of 92.4:7.6 (84.8% ee). The synthesis of this dipeptide was repeated, as above, using Carpino's amide bond forming conditions (HATU, HOAt, collidine). This synthesis yielded material with a relative peak size of at least 95:5 (>90% ee).

Ac$Y_sEY_sLDY_sDF$: The peptide was synthesized on 0.025 mmol scale by standard coupling procedures. Commercially available pre-loaded resin (0.5 mmol/g) was used. The N-terminal Fmoc group was cleaved and the amino group acetylated. The azidomethylene group was cleaved in the usual way. Sulfation was performed as described above. Cleavage and lyophilization affords 28 mg of a white solid. HPLC purification [Alltech Econosil C18, one major peak: t_2 = 22.76 min, gradient system: CH_3CN/0.1 M aq. NH_4OAc 5% – 75% in 40 min, 8 mL/min] affords 8 mg (27% based on resin loading, minus resin for characterization) of a flocculent white solid. **IR** (KBr): 1244 br, str, 1050 br, str; **LRMS** (MALDI, α-cyano-4- hydroxycinnamic acid matrix, negative ion mode): m/z 1138.3 [calc'd M-3SO_4+NH_4^+ 1138.48].

AcYEYLDYDF: A fraction of the phenol-deprotected material (10 mg resin) from the synthesis of $AcY_sEY_sLDY_sDF$ above was cleaved (yield 6 mg), dissolved in $MeOH/H_2O$ (2 mL) and subjected to hydrogenation over Pearlman's catalyst (10 mg) for 12h under an H_2 filled balloon. Filtration through pre-rinsed Celite ($MeOH/H_2O$, 1:1 eluant) afforded 3 mg crude material after lyophilization. **LRMS** (FAB α-cyano-4-hydroxycinnamic acid matrix, positive ion mode): m/z 1215.4 [calc'd MH+ $2Na^+$ 1215.44].

$AcYEY_2LDYDF$: The solid phase synthesis was performed according to the general procedures described above. Cleavage from the resin gave 17 mg of crude peptide. This material was subjected to hydrogenation over Pearlman's catalyst (20 mg) for 12h under an H_2 filled balloon. Filtration through pre-rinsed Celite (H_2O eluant). This material was subjected to HPLC (Alltech Econosil C18) gave three major peaks, two of which appeared to be deletion peptides (by MALDI-MS, we were unable to assign a structure based on the mass spectra, however the peptides appeared to be sulfated, as judged by HPLC retention time). The longest retained peptide (t_r = 33.48 min, gradient system: CH_3CN/~~1~~ 0.1 M aq. NHqOAc 5% – 75% in 40 min, 8 mL/min] pooling of this HPLC fraction and lyophilization afforded the desired peptide as a fluffy white solid (4.6 mg, 5.2 %) **LRMS** (MALDI, α-cyano-4-hydroxycinnamic acid matrix, negative ion mode): *m/z* 1170.4 [calc'd M-SO_4^-+NH_4^+ 1169.42]; (MALDI, 2,4,6- trihydroxyaceto-phenone, negative ion mode): *m/z* 1191.6 [calc'd M – SO_3 + Na^+ for $C_{57}H_{68}N_8O_{22}S$ 1191.41] **IR** (KBr): 1256 br, str, 1049 br, str.

$AcY_{Bn}E_{Bn}YLD_{Bn}Y_{Bn}D_{Bn}F$: After azidomethylene deprotection of the above peptide-resin a small portion was cleaved (8 mg resin) to yield ca. 2.5 mg of intermediate crude peptide. **LRMS** (FAB, α-cyano-4-hydroxycinnamic acid matrix, positive ion mode): m/z 1642.6 [calc'd MH + Na^+ 1642.69]. Other lower molecular weight peaks were observed, but were not assignable.

Synthesis of peptides by stepwise coupling of FmocTyr(SO_3Na)OH: Syntheses incorporating Fmoc Tyr(SO_3Na) in stepwise fashion were performed manually. Solvent volumes for the coupling steps and for washes are at least 10 mL per gram of resin. For coupling to non-sulfated tyrosine residues: Amino acid (3 equivalents relative to loading

capacity) is dissolved in one half of the total amount of NMP. PyBOP (3 equivalents relative to loading capacity) is dissolved in one quarter of the total amount of NMP. The resin is rinsed with one eighth of the total amount of NMP. Collidine (TMP, 9 equivalents relative to the loading capacity) is then added to the resin, followed by the amino acid solution and then the PyBOP solution. The resin is agitated with a nitrogen stream for 90 minutes, then all reagents are removed fro m the resin under vacuum. The resin is washed with NMP (4X) and MeOH(4X). Coupling completion is assessed using the Kaiser test.

Coupling of sulfated tyrosine residues:

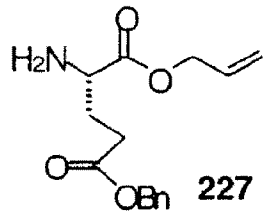
Amino acid (3 equivalents relative to loading capacity) is dissolved in one half of the total amount of NMP. HATU (3 equivalents relative to loading capacity) is dissolved in one quarter of the total amount of NMP. The resin is rinsed with one eighth of the total amount of NMP. HOAT 0.5M in NMP (1 equivalent relative to the loading capacity) is added to the resin, followed by collidine (TMP, 9 equivalents relative to the loading capacity), the amino acid solution and then the HATU solution. The resin is agitated with a nitrogen stream for 90 minutes, then all reagents are removed from the resin under vacuum. The resin is washed with NMP (4X) and MeOH (4X). Coupling is monitored using the Kaiser test.

Cleavage of sulfated peptides from chlorotrityl resin:

The resin is dried under high vacuum for two hours before the cleavage reaction is attempted. Dichloromethane/trifluoroethanol/acetic acid cleavage solution (7:2:1 v:v:v, 10mL per gram of resin) is cooled to 0 °C and added to a flask containing dried resin in an ice bath. The mixture is stirred for 1.5 hours at 0 °C. During this time the temperature does not exceed 5 °C. At the end of the reaction time the free peptide is filtered into a flask. The resin is then washed with the same volume of cleavage solution (at 0 °C) used in the reaction. Both washes are combined and most of the solvent is evaporated on a rotary evaporator (water bath less than 10 °C). Ether (40 mL) is added to the residue, the peptide is pelletted on the centrifuge and the ether is decanted. This procedure is repeated for another ether wash (40 mL) and for an ethyl acetate/ ether

wash (1.5:1 v:v, 25 mL total). The peptide pellet is redissolved in methanol, transferred to a flask, and evaporated to an oil (rotary evaporator water bath less than 10 °C). The oil is redissolved in methanol and evaporated to remove acetic acid. The oil is then lyophilized twice from MQ water to remove any traces of acetic acid. After removal of acetic acid, the crude peptide is stored at -25°C until HPLC purification.

L-Glutamic acid γ-benzyl-α-allyl ester:



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N-α-Fmoc-L-glutamic acid γ-benzyl ester-α-allyl ester was taken up in CH_2Cl_2/DMF (1:1, 20mL) and piperidine (7.5 mL) was added neat. After 25 minutes TLC analysis (2:1 Hex/EtOAc) indicated complete consumption starting material. The volume was reduced to ca. 2 mL in vacuo, dry Et_2O was added (30 mL) followed by concentrated HCl. A precipitate formed immediately and was filtered, washed with Et_2O and dried. The free base was obtained by dissolution in H_2O, neutralization with aqueous $NaHCO_3$ solution, extraction with EtOAc and drying *in vacuo*. This material was suitably pure for the next step. Analytically pure material was obtained by flash silica gel chromatography (of the free base 20 % MeOH-$CHCl_3$). **^{1}H NMR** (300 MHz, CDC1y): δ 7.35 (a s, 5H), 5.96-5. 87 (m, 1H), 5.36 (dd, J = 1.5 Hz, J = 7. 1 Hz, 1H), 5.28 (dd, J = 1.4 Hz, J = 10.0 Hz, 1H), 5.13 (s, 2H), 4.62 (a d, J = 5.7 Hz, 2H), 3.50 (dd, J = 5.1, J = 7.6 Hz, 1H), 2.53 (t, J = 7.5, 2H), 2.18-1.84 (m, 2H).

Fmoc Y_sE_{Bn}OH: Fmoc-L-glutamic acid γ-benzyl ester was attached to 2-Clt resin in the usual way. The resin loading was determined to be 0.75 mmol/g by quantitation of Fmoc cleavage. Resin (450 mg, 0.34 mmol) was subjected to the usual Fmoc cleavage conditions. FmocTyr(SO_3Na) was coupled using the general HATU-mediated coupling conditions described above [2 x (450 mg, 0.7 mmol)] with an extended coupling time

(5h). Cleavage of the dipeptide from the resin using the general cleavage conditions afforded suitably pure dipeptide (yield 67%) as assayed by ^{1}HNMR and analytical HPLC [gradient 25:75 CH_3CN/0.1 M aqueous NH_4OAc – 75:25/30 min, at 3 mL/min; retention time: 24.7 min, Vydac C18]. **^{1}H NMR** (300 MHz, CD_3OD): δ 7.75 (d, J = 7.4, 1H), 7.56-7.21 (m, 17H), 5.03 (d, J = 2.6 Hz, 1H), 4.90 (s, 2H), 4.49 (aq, J = 4.8 Hz, 1H), 4.32 (td, J = 5.9, 2.6 Hz), 4.25-4.10 (m, 3H), 3.12-2.68 (m, 2H), 2.47-2.42 (m, 2H), 2.22-2.18 (m, 1H), 1.98-1.91 (m, 1H).

Fmoc$Y_SE_{Bn}Y_SLD_{Bn}Y_SD_{Bn}$F: The stepwise procedure was employed as described above for the first six residues. The hexapeptide was α-amino deprotected as usual. The dipeptide, FmocY_SE_{Bn}OH (2.3 equivalents), was then coupled to the support-bound hexapeptide using HATU and HOAt (5h). Cleavage of the octapeptide was accomplished in the usual way. HPLC [Econosil C18, gradient 25:75 CH_3CN/~~.1~~ 0.1 M aqueous NH_4OAc – 75:25/30 – 40min at 8 mL/min; retention time: 25.4 min] (11% yield based on resin loading). **^{1}HNMR** (300 MHz, CD_3OD) is consistent with the structure.

pEYyLDYDF: This peptide was generated in the attempted synthesis of Fmoc $Y_SEY_SLDY_SDF$ *via* the stepwise protocol described above. HPLC purification of the product and analytical characterization revealed the pyroglutamate-terminated structure. A satisfactory mass spectrum was not obtained for this compound. However, 2-dimensional ^{1}HNMR analysis (TOCSY, COSY) showed, unambiguously, this sequence. HPLC [Econosil C18, gradient 25:75 CH_3CN/0.1M aqueous NH_4OAc – 75:25/30 – 40min at 8 mL/min; retention time: 36.8 min].

Those of ordinary skill in the art will recognize that materials, methods and procedures, including among others: starting materials, reagents, solvents, resins, reaction conditions, side- chain protecting groups, a-amino protecting groups, and carboxyl protecting groups, other than those specifically disclosed herein can be employed in the practice of this invention without resort to undue experimentation. Functional equivalents of materials, methods and procedures employed in the examples herein are known in the art and are intended to be encompassed by this invention.